

Review article

CD28/CTLA-4–CD80/CD86 and ICOS–B7RP-1 costimulatory pathway in bronchial asthma

Costimulatory molecules are cell surface glycoproteins that can direct, modulate and fine-tune T-cell receptor signals. The B7-1/B7-2–CD28/CTLA-4 and ICOS–B7RP-1 pathway provides key second signals that can regulate the activation, inhibition and fine-tuning of T-lymphocyte responses. The expression of B7-1/B7-2–CD28/CTLA-4 molecules on clinical samples from patients with asthma have been well studied, and the results indicate that different extents of these molecules are expressed on the surface of various cells, and that the concentrations of soluble form of these molecules are elevated in the sera of patients with asthma. There is a burst of papers describing an important role for B7-1/B7-2–CD28/CTLA-4 pathway in the Th1/Th2 balance. Similarly, ICOS stimulates both Th1 and Th2 cytokine production but may have a preferential role in Th2 cell development. Moreover, The B7-1/B7-2–CD28/CTLA-4 and ICOS–B7RP-1 pathway has been suggested of being involved in the development of airway inflammation and airway hyperresponsiveness. Further study of the functions of the pathways within the CD28/CTLA-4–CD80/CD86 and ICOS–B7RP-1 superfamily individually and their interplay should provide insights into the pathogenesis of asthma, and has great therapeutic potential for treatment of asthma.

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Bronchial asthma is prevalent worldwide, especially in developed countries where its prevalence is increasing to epidemic proportions (1). Patients with asthma suffer from acute bronchoconstriction and mucus formation directly after inhalation of the allergen. Chronic symptoms include airway hyperresponsiveness (AHR) to bronchospasmogenic stimuli, inflammation and airway remodelling. Central to the process of airway inflammation is the T lymphocyte (2). Thus, T-cell recruitment and differentiation are critical elements in the evolution of the asthmatic state. The factors that regulate these aspects of T-cell function are incompletely understood, but may include engagement of specific accessory molecules on the T cell. Production of Th2 cytokines, such as interleukin (IL)-4, IL-5 and IL-13, in asthma is at least partly responsible for eliciting the cardinal pathogenic changes of the asthmatic phenotype (3). The factors in asthma that govern the production of Th2 cytokines over Th1 cytokines, such as interferon (IFN)- γ , are slowly being revealed. One likely factor is the cytokine profile of T cells, which is influenced by antigen-presenting cells (APCs), possibly through costimulatory signals. Such signals may be provided by ligation of CD28 or cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) with B7-1 (CD80) or B7-2 (CD86; 4).

The term anergy was first coined by Von Pirquet in 1908 to describe the loss of delayed type hypersensitivity to tuberculin in individuals infected with measles virus. The term was clinically accepted since then to describe negative tuberculin skin test results in conditions, where it is expected to be positive. In 1980, the term anergy was used to describe the specific inactivation of B cells in mice by high doses of antigen. It was subsequently used for T cells to describe a phenomenon in which antigen presentation to T-cell clones in the absence of professional APCs induced a hyporesponsive state affecting subsequent IL-2 production and proliferation upon restimulation. It is well known that naive T lymphocytes require two distinct signals from APCs to be functionally activated (5, 6). The first signal which confers specificity, is provided by the interaction of the T-cell receptor with major histocompatibility complex II complexes on APCs. A second costimulatory signal can be provided by APC-borne ligands for the CD28 and CTLA-4 receptors on T cells. The known costimulatory molecules on T cells and their ligands on APCs are listed in Table 1.

Inhibition of T cell costimulatory molecules at the cell surface or their intracellular signal transduction has been repeatedly reported to play an important role in T-cell unresponsiveness. The interaction between B7 and CD28 may determine whether a T-cell response develops.

Table 1. Costimulatory molecules on T cells and their ligands on antigen-presenting cells

Costimulatory molecules	Ligands	Expression patterns
CD28	CD80 (B7-1) CD86 (B7-2)	CD28 is expressed on most mouse T cells, 90% of human CD4 ⁺ T cells and 50% of human CD8 ⁺ T cells. CD80 and CD86 are expressed on professional antigen-presenting cells, such as dendritic cells, macrophages and activated B cells, belong to the immunoglobulin superfamily and might exist as dimers and monomers, respectively.
CTLA-4	CD80 CD86	CTLA-4 is expressed on activated T cells, is ~30% homologous with CD28 and binds to the same ligands as CD28, albeit with a much higher affinity.
ICOS	B7RP-1 (B7-H2)	ICOS is the third member of the CD28 superfamily that shares about 20% homology with CD28. ICOS is not present on naïve T cells; instead it is up-regulated after T cell activation and retained on many memory T cells. B7RP-1 is expressed on B cells and macrophages.
PD1	B7-H1 (PDL1) B7-DC (PDL2)	PD1 is expressed by T cells, B cells, and myeloid cells. B7-H1 is expressed on dendritic cells, macrophages, T and B cells, endothelial cells, epithelial cells, muscle cells and trophoblast blast cells. B7-DC is expressed on dendritic cells and macrophages.
Not determined	B7-H3	B7-H3 is expressed on dendritic cells, macrophages and T cells. Potentially endothelial cells and epithelial cells.
BTLA	B7-H4	BTLA is expressed on Th1 cells and B cells. B7-H4 is expressed on dendritic cells, macrophages B cells, and T cells. Potentially endothelial cells and epithelial cells.

BTLA, B and T lymphocyte attenuator; CTLA-4, cytotoxic T lymphocyte associated antigen-4; ICOS, inducible costimulatory molecule; PD1, programmed cell death 1; Th1, T helper 1.

Blocking monoclonal antibody (mAb) to CD86 inhibit the development of specific immunoglobulin (Ig) E, pulmonary eosinophilia and AHR in mice. CTLA-4 seems to act on endogenous inhibitor of T-cell activation and CTLA4-Ig, a soluble fusion protein has also been effective in blocking AHR. Furthermore, cytokine production by memory/effector T cells, particularly those of Th2 subset, is highly dependent on costimulation via the inducible costimulatory molecule (ICOS)/B7RP1 (ICOS–ligand) pathway. Blockage or genetic disruption of this costimulatory pathway markedly reduced allergen-induced asthma in mice suggesting a promising approach for the treatment of allergic disease in humans. Another mechanism of direct T-cell suppression is inhibition of CD28 costimulation by IL-10. The IL-10 significantly inhibits the anti-CD28 costimulation (7). Thus, IL-10 suppresses only those T cells that have low numbers of T-cell receptors triggered and which require CD28 for proliferation. Ligation of IL-10 receptor at the time of CD28 stimulation inhibits tyrosine phosphorylation of CD28. As a consecutive event for signal transduction, the association of CD28 with the phosphatidylinositol 3-kinase p85 molecule is inhibited by IL-10.

The biological activity of the costimulatory molecules CD28 has been extensively studied. CD28 is constitutively expressed by T cells and interacts with the B7 molecules CD80 and CD86 (8, 9). This interaction results in an increased T-cell proliferation, IL-2 production and resistance to apoptosis. T cells that bind antigen and do not receive a costimulatory signal are thought to die or to become anergic (10, 11). CTLA-4 was initially described as a classical type I glycoprotein on the surface of activated T cells (12). CTLA-4 plays an important role in the downregulation of T-cell response, in T-cell homeostasis and in maintenance of peripheral tolerance. The CD28 homologue ICOS is upregulated on T cells after

activation (13, 14). The ICOS and CD28 have unique and overlapping functions that synergize to regulate CD4⁺ T cell differentiation. Like CD28, ICOS augments T-cell differentiation and cytokine production and provides critical signals for immunoglobulin production (15, 16).

Expression of B7 molecules in patients with asthma

Because B7 molecules CD80 and CD86 and related B7 homologues are especially significant on APCs for delivering requisite costimulatory signals to lymphocytes, The expression of B7 molecules on clinical samples from patients with asthma have been well studied (Table 2). In a early study, Hofer et al. (17) have demonstrated that atopic patients with asthma who are exposed to allergens have significantly higher levels of CD86 expression on B cells than atopic asthmatic subjects not exposed to allergen *in vivo* or nonatopic control subjects. In contrast, there were no differences in CD80 expression among the three study subject groups. When peripheral blood

Table 2. Expression of CD80/CD86–CD28/CTLA-4 molecules in patients with asthma

Molecules	Comments
CD80/CD86	Expression of membrane CD86, but not CD80, on B cells and macrophages is higher in atopic asthmatic patients; expression of membrane CD86 is upregulated following allergen challenge in asthmatics. Soluble CD86 concentration is elevated in sera of asthmatics, and serum sCD86 level is correlated with asthma severity
CD28/CTLA-4	Both soluble CTLA-4 and soluble CD28 concentrations are elevated in children and adult patients with allergic asthma

CTLA-4, cytotoxic T lymphocyte-associated antigen 4.

mononuclear cells from asthmatic patients or normal control subjects were stimulated with IL-4 or IL-13, the expression of CD86, but not CD80, was significantly increased on B cells. Cytoflowmetric analysis revealed that alveolar macrophages from asthmatics, unlike those from normal subjects or patients with pulmonary sarcoidosis or extrinsic allergic alveolitis, overexpressed CD86, to a lesser extent, CD80 surface molecules (18). However, Burastero et al. (19) have found that allergic individuals had a significantly higher proportion of alveolar macrophages expressing the CD80 molecule than control subjects, whereas no difference was observed in CD86 expression. In addition, in asthmatic subjects high level of CD80 expression on alveolar macrophages did not change following allergen challenge; in contrast, CD86 membrane expression was upregulated following allergen challenge (20).

Numerous studies have demonstrated that soluble (s) forms of B7/CD28 molecules have potent functional activity and that, depending on the experimental system used, can either inhibit or enhance immune responses (21–24). The sCD86 might play an important role in immune regulation by binding with the CD28 molecules, thus, interfering with the binding of CD28 and/or CTLA-4. It has been reported that sCD86 results from an alternatively spliced transcript (CD86deltaTM) characterized by deletion of the transmembrane domain (21). Recombinant CD86deltaTM binds to CD28 and CTLA-4 and induces the activation of T cells after stimulation with anti-CD3 mAb. CD86deltaTM also induces IFN- γ production by virus-specific CD8⁺ memory human T cells stimulated with the Flu M1 peptide (21). The sCD86 molecule, therefore, appears to be a functional costimulatory molecule playing a potentially important role in immune surveillance. In a recent study (25), we have shown that sera from patients with acute asthma exacerbation had much higher levels of sCD86 than sera from stable asthmatics and healthy individuals, and there was no difference between the two latters. In asthmatics, the serum sCD86 level was correlated with AHR, forced expiratory volume in 1 s, and with artery partial pressure of carbon dioxide. The other studies also demonstrated that plasma sCD80 and sCD86 concentrations are elevated in children and adult patients with allergic asthma (26, 27). These data suggest that B7 molecules might be involved in the mechanism of asthma, especially the acute exacerbation of asthma.

Despite widespread use of glucocorticoids to treat inflammatory diseases, the precise mechanism of action for this treatment remains uncertain. In the case of asthma treatment, glucocorticoids are delivered directly to the airway, and thus, immediate cellular targets include immune cells (e.g. T cells, macrophages, mast cells and eosinophils) and resident parenchymal cells (e.g. airway epithelial and smooth muscle cells). In these cell types, the glucocorticoid action appears to proceed via the

glucocorticoid receptor and its capacity to alter gene transcription (28). However, the precise cellular and molecular targets still need to be determined. In a more recent double-blind, placebo-controlled, parallel group study (29), we investigated the effect of oral prednisolone on the serum sCD86 concentrations in patients with allergic asthma, and our data showed that 2 weeks of oral prednisolone 30 mg/day reduced the serum concentrations of sCD86 in allergic asthma. It is known that glucocorticoids are effective in reducing airway inflammation through multiple mechanisms, including the modulation of cytokine and chemokine production in lymphocytes and endothelial cells (30). Glucocorticoids bind to specific receptors in the cytosol, are translocated to the nucleus, and bind to specific regulatory sequences of target genes (31). It has been reported that increased glucocorticoid therapy of asthmatics was associated with significant reductions in the percentages of peripheral blood mononuclear cell expressing mRNA encoding IL-5, IL-4 and IL-2 and an increase in those expressing IFN- γ mRNA (32). By regulating cytokine gene expression (33), glucocorticoids may critically modulate effector functions of T cells. The findings of our above study provided further evidence that glucocorticoids exert their therapeutic effects in the treatment of asthma by reducing serum sCD86 concentrations.

Membrane CD86 are expressed predominantly on monocytes, dendritic cells, lymphocytes, eosinophils and neutrophils (34–37). It has been demonstrated that the transcript for sCD86 is expressed in normal monocytes and dendritic cells (38). Our results also showed that the serum sCD86 level was positive correlated with numbers of lymphocytes, eosinophils and monocytes, but not neutrophils (25). These results suggested that sCD86 detected in asthmatic serum might derive from lymphocytes, eosinophils and monocytes. However, our *in vitro* experimental results showed that cultured T cells, B cells, neutrophils or eosinophils from either asthmatics or healthy volunteers did not produce detectable sCD86. In contrast, without any stimulation, monocytes were capable of releasing sCD86 into the culture supernatants, and monocytes from asthmatics produced more sCD86 than did those from healthy volunteers (25). Therefore, we inferred that serum sCD86 derived from monocytes in peripheral blood, and that monocytes were further responsible for the elevation of serum sCD86 in asthmatics with acute exacerbation. On the contrary, we could not rule out the possibility that other types of leucocytes such as lymphocyte and eosinophils would also be able to produce sCD86 *in vivo* under asthmatic conditions.

CD28/CTCA-4 and asthma

CD28 is a 44-kDa glycoprotein that binds to CD80 and CD86 on APCs, which is the best characterized and most

effective costimulatory molecule expressed by naive and primed T cells (39). CD28 costimulation has been implicated in a wide array of T-cell responses, including T-cell proliferation, IL-2 production, prevention of anergy and induction of the antiapoptotic factor Bcl-xL (39, 40). Furthermore, CD28 plays an important role in B-cell differentiation and antibody production by controlling T cell help provided to B cells and germinal center formation. Finally, CD28 also directs T-cell migration into inflammatory sites by inducing the production of specific chemokines and the regulation of chemokine receptors (41). CTLA-4 was initially described as a classical type I glycoprotein on the surface of activated T cells (12). CTLA-4 is a member of the immunoglobulin gene superfamily and along with its homologue, CD28, is a B7-binding protein (42). CTLA-4 plays an important role in the downregulation of T-cell response, in T-cell homeostasis and in maintenance of peripheral tolerance. CTLA-4 signalling blocks IL-2 production and expression of IL-2 receptor (43), and inhibits the function of cytolytic T cells (44). Different mechanisms of action of CTLA-4 have been recognized. One is delivery of a negative signal to the T cells, and another is B7 molecule sequestration on the APCs. Which of these two mechanisms dominate depends on the level of surface expression of CTLA-4. The two mechanisms may have distinct functional outcomes. The first results in rapid inhibition of T-cell activation, and the second leads to induction of T-cell anergy (45). The most compelling data that supports such a role for CTLA-4 comes from experiments in which the CTLA-4 gene is rendered inactive via construction of CTLA-4 knockout mice (46). Such mice demonstrate profound polyclonal lymphoproliferative disorders that infiltrate most major organ systems and die a few weeks after birth.

Howard et al. (47) have refined a region on chromosome 2q33 that contains one or more susceptibility genes for allergic responses, asthma, or both. By resequencing *CTLA4* and *CD28* genes in individuals from diverse ethnic groups, these authors have identified 14 new single-nucleotide polymorphisms (four in *CTLA4* and 10 in *CD28*) and evaluated a subset of the single-nucleotide polymorphisms for association with asthma and allergic phenotypes. Significant associations were observed with polymorphisms in *CTLA4* that might regulate its expression levels, the function of the CTLA-4 protein, or both. Additionally, combinations of polymorphism genotypes within *CTLA4* increase the risk for increased total serum IgE levels (with +49 and 3'-UTR AT repeat) or asthma (with -1147 and +49) in this population. Lee et al. (48) have investigated the association between two polymorphisms of CTLA-4 (exon 1: +49 A/G, promoter: -318 C/T) and atopy, asthma severity and AHR, in asthmatic patients and a healthy control group. In that study, the CTLA-4 promoter (-318 C/T) polymorphism was found to be associated with asthma severity, but not with asthma, atopy, or AHR. The CTLA-4 exon 1 (+49

A/G) polymorphism was shown to be associated with AHR, but not with asthma, atopy, or asthma severity. From these results, it appears that the CTLA-4 promoter (-318 C/T) T allele may serve as a clinically useful marker of severe asthma, and the CTLA-4 exon 1 (+49 A/G) polymorphism may have a disease-modifying effect in asthmatic airways. It has also been reported that significant associations between *CTLA4* polymorphisms and serum IgE levels, allergy, allergic asthma, asthma and reduced lung function, but not AHR, suggesting that a major contribution of the polymorphisms is to atopy. Haplotype analysis suggests that one or more of the newly identified polymorphisms MH30, CT60, JO31, JO30 and JO27_1 could be involved in the development of allergic disease (49). All associated alleles were opposite those alleles associated with autoimmune diseases, providing suggestive evidence for a genetic control site for Th1-mediated vs Th2-mediated immunity within the *CTLA4* gene. Recent evidence indicates that the -109 C/T polymorphism at the FCER1B promoter region affects total serum IgE levels in asthmatic patients (50). Another study suggests that promoter polymorphisms of both CTLA-4 and FCER1B are genetic factors that influence total serum IgE levels in patients with asthma (51).

The sCTLA-4 might play an important role in immune regulation by binding with the B7 molecules, thus, interfering with the binding of CD28 and/or CTLA-4. *In vivo* experiments with mutant lacking the cytoplasmic domain of CTLA-4 indicated that its inhibitory effects are mediated in part through the ability of the extracellular domain to compete for ligands (52). Recombinant sCTLA-4 at high concentrations *in vitro* could inhibit mixed leucocyte reactions and cellular proliferation (53), probably by interference with CD28 signalling. The sCTLA-4 might also interfere with CTLA-4-B7 interactions and block negative signals transmitted via surface-bound CTLA-4. Our recent data showed that serum sCTLA-4 concentrations in both atopic asthmatic and nonatopic asthmatics were all higher than that in normal controls. There was no difference in serum sCTLA-4 concentrations between atopic and nonatopic asthmatics. The serum sCTLA-4 concentrations in the asthmatics statistically correlated with forced expiratory volume in 1 s, percentage of predicted peak expiratory flow, and with artery partial pressure of carbon dioxide, respectively. Our data also showed that the concentration of sCTLA-4 was significantly related to blood lymphocyte numbers (54). The other studies also demonstrated that plasma sCTLA-4 and sCD28 concentrations are elevated in children and adult patients with allergic asthma (26, 27). Like serum sCD86 (23), our recent study further showed that serum sCTLA-4 concentrations increased after allergen inhalation in sensitized asthmatic subjects, and that serum sCTLA-4 concentrations were downregulated by prednisolone therapy (55).

B7-CD28/CTLA-4 and Th1/Th2 imbalance

It is well known that Th2 cells secrete IL-4, IL-5, IL-13, etc., which promote allergic inflammation and stimulate B cells to produce IgE and other antibodies. In contrast, Th1 cells produce IFN- γ and IL-2, which initiate the killing of viruses and other intracellular organisms by activating macrophages and cytotoxic T cells (56). Th1 cells may dampen allergic inflammation or efficiently contribute to the effector phase in allergic diseases depending on specific disease model and stage of the inflammation. Obviously there is clear evidence on epithelial apoptosis and IFN- γ and asthma and atopic dermatitis (57–59). These two subgroups of helper T cells arise in response to different immunogenic stimuli and cytokines, and they constitute an immunoregulatory loop: cytokines from Th1 cells inhibit Th2 cells, and vice versa. An imbalance in this reciprocal arrangement may be the key to asthma (60). It should be noted that Th1/Th2 imbalance cannot be observed in some mouse studies, and that might be related to innate immune response stimulating substance (lipopolysaccharide, etc.) contamination of one reagent, but not the other. Most of the mouse asthma models studies have been performed with lipopolysaccharide containing ovalbumin since 2 years ago.

Using an *in vitro* T-cell differentiation assay of T cells from a myelin basic protein transgenic mouse, Kuchroo et al. (61) have shown that anti-CD80 mAb increases the production of IL-4, whereas anti-CD86 mAb enhances the production of IFN- γ . Furthermore, the majority of Th clones derived from anti-CD80 mAb-injected mice were of the Th2 phenotype. This information was used to manipulate the two subsets selectively *in vivo* to affect the induction of the autoimmune disease encephalomyelitis. Administration of anti-CD80 mAb significantly reduced the incidence of encephalomyelitis, an effect prevented by simultaneous administration of anti-IL-4 mAb, whereas injection of anti-CD86 mAb substantially increased disease severity. Injection of anti-B7 mAbs did not significantly affect the induction of T cells to the immunizing antigen, but rather altered the cytokine profile of the responding T cells. These data suggest that treatment with anti-B7 mAbs can alter the course of disease by differentially influencing the development of mature Th1 and Th2 effector cells from uncommitted Th0 cells. Other studies also support an important role for CD86 in the signalling of IL-4 production and the development of Th2 cell (62).

Van Neerven et al. (63) have demonstrated that proliferation of peripheral blood T lymphocytes to Der p allergen and the recall antigens tetanus toxoid, and *Candida albicans* was strongly inhibited by CTLA4-Ig. The inhibition of proliferation by CTLA4-Ig was partially overcome by stimulating the T lymphocytes with a CD28-specific mAb, indicating that CTLA4-Ig inhibits interaction of CD28 with CD80 and CD86. CD86-specific

mAb IT2.2 inhibited the response to antigens in a similar way as CTLA4-Ig, but the degree of inhibition with anti-CD86 mAb was slightly less than with CTLA4-Ig. In addition to the inhibition of proliferative responses of allergen-specific T cells, the allergen-specific production of mRNA for the Th1 cytokines IL-2 and IFN- γ , but also Th2 cytokines IL-4 and IL-5, was inhibited by CTLA4-Ig treatment. The data presented here suggest that inactivation of allergen-specific T-cell responses (including Th2-like responses) can be achieved by blocking costimulatory signals. Another study also showed that peripheral blood mononuclear cell proliferation and IL-5 and IL-13 production to allergen stimulation is inhibited by CTLA4-Ig fusion protein and by mAbs to CD86, but not CD80, and that antigen presentation by airway APC and allergen-induced proliferation from airway T cells is CD86-dependent (64). CD86 has been further confirmed to be the primary B7 signalling homologue in Th1/Th2 cytokine production of human peripheral blood mononuclear cells, and second signal pathways through the B7 homologues have no effect on phenotypically differentiated T-helper cells in humans (65).

The studies by Jaffar et al. (66) have further demonstrated that *ex vivo* allergen stimulation of bronchial mucosal tissue from mild atopic asthmatics, but not atopic nonasthmatic control, volunteers elicited an increase in the production of IL-4, IL-5 and IL-13. There was no production of IFN- γ or granulocyte-macrophage colony-stimulating factor by the bronchial explants from both asthmatic and control subjects. Interestingly, neutralizing mAbs to CD80 blocked allergen-induced cytokine secretion by the asthmatic tissue, and mAbs to CD86 were only marginally less effective at inhibiting the response. In addition, the allergen-induced secretion of IL-5 and IL-13 by the bronchial explants from asthmatics could be inhibited by CTLA4-Ig (67). Similarly, allergen-induced production of IL-5 and IL-13 by peripheral blood mononuclear cell could also be inhibited by CTLA4-Ig (68). Therefore, blockade of the interaction of B7 costimulatory molecules with CD28 on T cells using specific immunological agents may provide a novel therapeutic intervention in the treatment of allergic inflammatory diseases such as asthma.

CD4⁺CD25⁺ regulatory T cells are important components of the homeostasis of the immune system, as impaired CD4⁺CD25⁺ T-cell activity can cause autoimmune diseases and allergic diseases, including asthma (69). CD4⁺CD25⁺ T cells play a key role in regulating airway eosinophilic inflammation, possibly by suppressing Th2 responses to allergens (69). Recent work indicates that CD28 : B7 interactions are needed for development and maintenance of CD4⁺CD25⁺ T cells, which play an essential role in regulating self-tolerance and T-cell activation (70, 71). It has been demonstrated that transmission of a suppressive signal by CD4⁺CD25⁺ T cells requires engagement of the B7 molecule expressed on target T cells. CD80/CD86^{-/-}

CD4⁺CD25⁻ T cells were resistant to suppression by concentrations of CD4⁺CD25⁺ T cells that suppressed wild-type CD4⁺CD25⁻ T-effector cells (72). Zheng et al. (73) have demonstrated that during T-cell responses to alloantigens expressed on dendritic cells, mAbs against CD86 potentially enhanced suppression by CD4⁺CD25⁺ T cells. In contrast, blocking CD80 enhanced proliferative responses by impairing suppression of CD4⁺CD25⁺ T cells. Intriguingly, the relative expression levels of CD80 and CD86 on dendritic cells are modulated during progression from an immature to a mature state, and this correlates with the ability of CD4⁺CD25⁺ T cells to suppress responses. These data show that CD80 and CD86 have opposing functions through CD28 and CTLA-4 on CD4⁺CD25⁺ T cells.

ICOS/B7RP-1 costimulation

The ICOS is the third member of the CD28 superfamily. The ICOS is a homodimer of 55–60 kDa that shares about 20% homology with CD28 (74). However, unlike CD28, ICOS is not present on naive T cells; instead it is upregulated after T-cell activation and retained on many memory T cells (14, 74). The ICOS binds its own B7 family member, B7RP-1 or B7h, on B cells and macrophages (13, 75). The ICOS can induce CD28-independent T-cell expansion and selective cytokine production (13, 74). Data from ICOS^{-/-} mice have shown that this molecule plays a critical role in T–B cell interactions, is essential for germinal center formation and humoral immune responses and delivers a key signal for IL-4, but not IFN- γ production (76, 77). Engagement of ICOS is particularly effective in costimulating IL-10 and IL-4, but not IL-2 production. A series of studies have supported the concept that ICOS functions as a critical costimulatory pathway for Th2 responses (78).

In a model of allergic airway disease, to test whether ICOS/B7RP-1 interactions are required for promoting Th2 inflammatory responses, Tesciuba et al. (79) gave mice an intraperitoneal injection of ICOS-Ig, a soluble form of ICOS, during sensitization and challenge. In this system, the authors have found that blockade of ICOS-mediated costimulation dramatically reduced cellular infiltration of cells into the airways of inflamed lungs, and that *ex vivo* restimulation of T cells from the inflamed lungs of ICOS-Ig-treated mice resulted in the production of significant amounts of the Th2 cytokine IL-5. There was no increase in the Th1 type cytokine IFN- γ , which remained below the level of detection. Interestingly, unlike CTLA4-Ig treatment, no change in T-cell priming or differentiation was observed when mice are treated with ICOS-Ig. Cytokine production from restimulation of *ex vivo* Th2 effector lung cells was inhibited by ICOS-Ig. These results suggest that ICOS-mediated costimulation may regulate Th2 effector cell function without affecting Th2 differentiation to a Th2 type antigenic stimulation.

Another study has shown that ICOS stimulation enhanced proliferation of CD4⁺ cells and production of IFN- γ , IL-4 and IL-10, but not IL-2. When the ICOS : B7h interaction was blocked by ICOS-Ig, CD4⁺ T cells produced more IFN- γ and less IL-4 and IL-10 than CD4⁺ cells differentiated with control immunoglobulin (80). These results demonstrate that ICOS stimulates both Th1 and Th2 cytokine production but may have a preferential role in Th2 cell development. These data suggest that CD80/CD86 costimulation may be needed to optimally upregulate ICOS expression and that the CD28 pathway may exert some of its effects (such as Th2 differentiation) via ICOS. More recently, it has been shown that transfer of the ICOS-enriched T-cell population followed by allergen airway challenges induced pronounced infiltration of recipient T and B cells and local production of allergen-specific IgE by intrapulmonary plasma cells. In contrast, transfer of the ICOS-depleted T-cell fraction resulted in the recruitment of significantly lower numbers of B cells and no local IgE production (81). These data indicate that expression of ICOS defines a subset of T-effector cells that are required for B-cell infiltration and local IgE production in lung tissues on allergen airway exposure.

Gonzalo et al. (82) have compared the roles of ICOS and CD28 in regulating Th2-mediated mucosal inflammatory responses. It was found that CD28 is critical during priming but does not contribute to recall responses once effector Th2 cells have been generated. In contrast, ICOS plays the predominant role in regulating Th2 effector cell activation. The ICOS-mediated signalling contributes to the inflammatory response not only through the regulation of IL-4 but also by providing a signal for upregulation of the chemokine receptors CCR3, CCR4 and CCR8. These data suggest a scenario whereby both molecules regulate the outcome of the immune response but play separate key roles: CD28 primes T cells and ICOS regulates effector responses. Consistent with the observations of others (79, 82), Wiley et al. (83) have also shown that blockade of the ICOS/B7RP-1 pathway in mice during initial encounter with antigen attenuates Th2-polarized allergic airway inflammation, but does not alter systemic features of sensitization. In that study, intraperitoneal treatment of mice with anti-ICOS neutralizing mAb during sensitization resulted in a marked reduction in airway eosinophilia and IL-5 in bronchoalveolar lavage, but had no effect on IL-4, IL-13 and eotaxin content in bronchoalveolar lavage or the production of antigen-specific IgE in serum. Cultured splenocytes from mice sensitized to the same antigen in the context of ICOS ablation produced enhanced levels of IL-4 and IL-5 upon stimulation with antigen, and this correlated with elevated inflammation and IgE secretion upon long-term *in vivo* antigen recall; the deleterious effects ICOS blockade, however, were not associated with reduced IL-10 production by splenocytes. Peculiarly,

anti-ICOS intervention during antigen rechallenge had no effect on airway inflammation or immunoglobulin production, despite high levels of ICOS expression on infiltrating CD4⁺ T cells. Therefore, these data challenge the notion that the ICOS/B7RP-1 costimulatory axis represents a promising therapeutic target for immune-inflammatory disorders such as asthma. Although neutralization of ICOS during sensitization and acute antigen exposure attenuated airway inflammation, this intervention ultimately exacerbated immune-inflammatory phenomena upon long-term *in vivo* recall. Moreover, blockade of ICOS signalling during rechallenge of mice with established allergic disease elicited no therapeutic effect, intimating that ICOS may be functionally redundant in mature memory responses.

Recent studies indicated that CD4⁺CD25⁺ T cells operate directly in the autoimmune lesion and are dependent on ICOS to keep it in a nondestructive state (84). The works by Kohyama et al. (85) and colleagues have shown that IL-10-producing regulatory T cells expressed high levels of ICOS before *in vitro* stimulation. Blockade of ICOS signalling inhibited the production of IL-10 and abrogated the inhibitory function of these regulatory T cells. Thus, ICOS signal plays a critical role in immune regulation by IL-10-producing T-regulatory cells in self-tolerance. It has been reported that mature pulmonary dendritic cells in the bronchial lymph nodes of mice exposed to respiratory allergen induced the development of T-regulatory cells, in a process that required T-cell costimulation via the ICOS-ICOS-ligand pathway. When adoptively transferred into sensitized mice, the IL-10-producing T-regulatory cells blocked the development of AHR. Both the development and the inhibitory function of regulatory cells were dependent on the presence of IL-10 and on ICOS-ICOS-ligand interactions (86). These studies demonstrate that T-regulatory cells and the ICOS-ICOS-ligand signalling pathway are critically involved in respiratory tolerance and in down-regulating pulmonary inflammation in asthma.

Effects of CD28/CTCA-4 on airway inflammation and AHR

It is well known that CD80 and CD86 are capable of forming homodimers, allowing for interactions with homodimers of either CD28 or CTLA-4. The interaction of CD28 with its ligands is weaker than the interaction with CTLA-4. Human CD80 binds to human CTLA-4 and CD28 with kDa values of 0.42 and 4 μ M, respectively for the monomeric interactions (87). The CD86 : CTLA-4 interaction is of an affinity similar to CD80 : CD28, and the CD28 : CD86 interaction is of even lower affinity (88). Despite having the same ligands, CD80 and CD86 appear to be involved in different mechanisms; CD80 can be more potent than CD86 in inducing an antitumoural response, while CD86 preferentially induces the production of a Th2 response (61, 89). Because of its constitutive

expression on human APCs, CD86 has been suggested of being involved in the initiation of the immune response.

By creating a mutant version of CTLA4-Ig that could selectively bind CD80 and block CD28-CD80 interaction but leave CD28-CD86 binding intact, Harris et al. (90) have demonstrated that CD80 blockade prevented antigen-induced accumulation of eosinophils and lymphocytes in the lung of immunized mice, but did not block antigen-induced systemic blood eosinophilia or IgE antibody production. No preferential expression of CD80 could be demonstrated on a population of lung APC consisting mainly of macrophages. These results indicated that CD80 costimulation is not necessary for the induction of Th2 immune responses but rather for the maintenance or amplification of lung inflammatory responses. In another study (91), these authors have further showed that CD80 costimulation is necessary for the induction of airway eosinophilia in immunized mice (14) is not a result of any defect in the accumulation of antigen-specific T cells at inflamed sites or in the draining lymphoid tissue. Instead, CD80 blockade decreased the production of the cytokines IL-4 and IL-5 by specific T cells following exposure to delivered soluble antigen. This decreased cytokine production is likely to result in decreased eosinophilia, as IL-5 production has been shown previously to be critical for the development of airway eosinophilia (92, 93). Interestingly, Mathur et al. (79) have demonstrated that CD28 interactions with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice (94).

Like human asthmatic patients (17, 18), allergen exposure upregulated expression of CD86, but not CD80, on B cells from murine lungs within 24 h (81). CTLA4-Ig treatment suppresses the production of IL-4, IL-5 and IL-10 from lung T cells. In contrast, pretreatment with CTLA4-Ig enhanced the production of IFN- γ from lung T cells by threefold. Thus, in the absence of costimulation through CD28 ligands, cells can default to a phenotype that produces less Th2 cytokines and more IFN- γ (95). Moreover, airway administration of an anti-CD86 mAb inhibited eosinophil infiltration, IgE production and Th2 cytokine secretion comparable in magnitude to that observed with CTLA4-Ig. Treatment with an anti-CD80 mAb had a small, but significant effect on eosinophil accumulation, although was less effective in inhibiting Th2 cytokine production. In addition, the anti-CD86, but not anti-CD80, mAb also inhibited antigen-induced AHR *in vivo*. We and other authors' studies also suggested that the CD86 costimulatory ligand plays a major role in the development of allergic inflammation and AHR in allergen-challenged mice (96-98), and that T-B cell interactions during allergic sensitization are amenable to therapeutic manipulation and that selective blockade of accessory signals can be an effective means for modulating distinct T-cell functions (97).

In BALB/c mice, Deurloo et al. (99) have shown that CTLA4-Ig significantly inhibited antigen-specific IgE in

serum, eosinophils in the bronchoalveolar lavage fluid, and IL-5 production by lung lymphocytes upon antigen restimulation *in vitro*. Importantly, CTLA4-Ig performed better than the gold-standard dexamethasone because this corticosteroid did not inhibit the upregulation of serum IgE. In a more 'severe' ongoing model, induced by sensitization to antigen emulsified in aluminium hydroxide, resulting in AHR and stronger inflammatory responses, CTLA4-Ig was less effective in that only the number of eosinophils in the bronchoalveolar lavage fluid was reduced, whereas dexamethasone inhibited both airway eosinophilia and cytokine production (99). Another also confirmed that in BALB/c mice, enhanced Th2 sensitization after anti-CTLA-4 mAb injections resulted in more severe airway eosinophilia and AHR, IL-4 and IL-5 levels in bronchoalveolar lavage fluid following repeated allergen inhalations. Importantly, aggravation of airway inflammation and enhancement of Th2 responses were accompanied by a significant reduction of pulmonary transforming growth factor (TGF)- β levels at protein level in bronchoalveolar lavage fluid as well as on mRNA level in inflamed lung tissue (100). In contrast to BALB/c mice, IgE production, airway inflammation and TGF- β production were not affected by administration of anti-CTLA-4 mAb in C57BL/6 mice (100). The presence or absence of CTLA-4-mediated TGF- β secretion may thus underline the difference in inflammatory responses between BALB/c and C57BL/6 mice after blockade of CTLA-4. Therefore, CTLA-4-mediated TGF- β production may represent a link between genetic predisposition and asthma development. The roles of CD28/CTLA-4-CD80/CD86 and ICOS-B7RP-1 costimulatory pathway in the development of experimental asthma are summarized in Table 3.

Mark et al. (101) have examined the roles of CD80 and CD86 in a model of allergic pulmonary inflammation and AHR by using mice with germline deletions of the CD80 and/or CD86 molecules. It was demonstrated that pulmonary eosinophilia, AHR and lung inflammation usually observed in mice sensitized and challenged with allergen are not present in CD80/CD86^{-/-} mice, suggesting that both CD80 and CD86 are indeed required for a normal asthma-like response to allergen in mice. These same disease parameters are also reduced in CD86^{-/-} mice and reduced to a lesser extent in CD80^{-/-} mice, suggesting that, although CD86 is quantitatively more important than CD80 in the induction of this response, CD80 and CD86 may have complementary functions in mediating the development of allergic pulmonary inflammation. Together, the above data support complementary roles for CD80 and CD86 in allergic pulmonary inflammation, with CD86 costimulation playing a quantitatively more prominent role. Burr et al. have also examined the role of CD28 and CTLA-4 in a murine model of allergic airway inflammation (102). CD28^{-/-} mice did not manifest airway inflammation in response to challenge, despite evidence of systemic sensitization. This was due to a

Table 3. *In vivo* studies of functions of B7/CD28 and ICOS-B7RP-1 in experimental asthma

Experimental models	Effects
CD80 blockade*	Decreases the production of IL-4 and IL-5 by specific T cells; prevents antigen-induced airway infiltrate of inflammatory cells, but does not block systemic blood eosinophilia or IgE production
CD86 blockade	Inhibits airway inflammation and airway hyperresponsiveness, IgE production and Th2 cytokine secretion
CD80/CD86 ^{-/-} mice	Pulmonary eosinophilia, airway hyperresponsiveness and lung inflammation cannot be induced by allergen in CD80/CD86 ^{-/-} mice
CTLA4-Ig treatment	Suppresses the production of IL-4, IL-5 and IL-10, and enhances the production of IFN- γ from lung T cells; inhibits antigen-specific IgE production
CD28 ^{-/-} mice	Does not manifest airway inflammation in response to challenge, despite evidence of systemic sensitization; prevention of CTLA-4 engagement by blockade of CD80 and CD86 in CD28 ^{-/-} mice restores lymphocyte recruitment to the airway but did not result in tissue eosinophilia, immunoglobulin isotype switching, or Th2 cytokine secretion
ICOS blockade	Attenuates Th2-polarized allergic airway inflammation, but does not alter systemic features of sensitization
ICOS-Ig treatment	Results in the production of significant amounts of IL-5, but not IFN- γ ; reduces cellular infiltration of cells into the airways of inflamed lungs
Transfer of the ICOS-enriched T cells	Induces pronounced infiltration of recipient T and B cells and local production of allergen-specific IgE by intrapulmonary plasma cells

*The blockade can be mediated by neutralizing monoclonal antibodies, small molecules that can compete for counter-receptor binding or methods that inhibit the expression of the molecules itself.

CTLA-4, cytotoxic T lymphocyte-associated antigen 4; ICOS, inducible costimulatory molecule; IFN, interferon; IL, interleukin; Ig, immunoglobulin.

failure in T-cell recruitment and defective Th2 cell development. Prevention of CTLA-4 engagement by blockade of CD80 and CD86 in CD28^{-/-} mice restored lymphocyte recruitment to the airway but did not result in tissue eosinophilia, immunoglobulin isotype switching, or Th2 cytokine secretion. However, the inflammatory infiltrate lacked eosinophils; thus, the inflammation observed in CTLA4-Ig-treated CD28^{-/-} mice was qualitatively different from that seen in wild-type mice (102). Thus, the processes of T-cell recruitment to the airway and T-cell differentiation have distinct requirements for signals mediated through the CD28 and CTLA-4 receptors, demonstrating that these receptors are important regulatory components in the development of allergic airway inflammation.

Conclusions

Patients with asthma suffer from acute bronchoconstriction and mucus formation directly after inhalation of the allergen. T-lymphocyte recruitment and differentiation are critical elements in the evolution of the asthmatic

state. The B7-1/B7-2-CD28/CTLA-4 and ICOS-B7RP-1 pathway provides key second signals that can regulate the activation, inhibition and fine-tuning of T-cell responses. The expressions of B7-1/B7-2-CD28/CTLA-4 molecules on clinical samples from patients with asthma have been well studied, and the results indicate that different extents of these molecules are expressed on the surface of various cells, and that the concentrations of soluble form of these molecules are elevated in the sera of patients with asthma. The B7-1/B7-2-CD28/CTLA-4 and ICOS-B7RP-1 pathway has been suggested of being involved in respiratory tolerance and in downregulating pulmonary inflammation in asthma. Current data suggest that inactivation of allergen-specific T-cell responses can be achieved by blocking costimulatory signals, which alters the course

of asthma by differentially influencing the development of mature Th1 and Th2 effector cells from uncommitted Th0 cells. Therefore, manipulation of the pathways within the CD28/CTLA-4-CD80/CD86 and ICOS-B7RP-1 superfamily individually and their interplay may provide a novel therapeutic intervention in the treatment of allergic inflammatory diseases such as asthma.

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